

ORIGINAL ARTICLE

Anti-Interleukin-12 Antibody for Active Crohn's Disease

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ABSTRACT

BACKGROUND

Crohn's disease is associated with excess cytokine activity mediated by type 1 helper T (Th1) cells. Interleukin-12 is a key cytokine that initiates Th1-mediated inflammatory responses.

METHODS

This double-blind trial evaluated the safety and efficacy of a human monoclonal antibody against interleukin-12 (anti-interleukin-12) in 79 patients with active Crohn's disease. Patients were randomly assigned to receive seven weekly subcutaneous injections of 1 mg or 3 mg of anti-interleukin-12 per kilogram of body weight or placebo, with either a four-week interval between the first and second injection (Cohort 1) or no interruption between the two injections (Cohort 2). Safety was the primary end point, and the rates of clinical response (defined by a reduction in the score for the Crohn's Disease Activity Index [CDAI] of at least 100 points) and remission (defined by a CDAI score of 150 or less) were secondary end points.

RESULTS

Seven weeks of uninterrupted treatment with 3 mg of anti-interleukin-12 per kilogram resulted in higher response rates than did placebo administration (75 percent vs. 25 percent, $P=0.03$). At 18 weeks of follow-up, the difference in response rates was no longer significant (69 percent vs. 25 percent, $P=0.08$). Differences in remission rates between the group given 3 mg of anti-interleukin-12 per kilogram and the placebo group in Cohort 2 were not significant at either the end of treatment or the end of follow-up (38 percent and 0 percent, respectively, at both times; $P=0.07$). There were no significant differences in response rates among the groups in Cohort 1. The rates of adverse events among patients receiving anti-interleukin-12 were similar to those among patients given placebo, except for a higher rate of local reactions at injection sites in the former group. Decreases in the secretion of interleukin-12, interferon- γ , and tumor necrosis factor α by mononuclear cells of the colonic lamina propria accompanied clinical improvement in patients receiving anti-interleukin-12.

CONCLUSIONS

Treatment with a monoclonal antibody against interleukin-12 may induce clinical responses and remissions in patients with active Crohn's disease. This treatment is associated with decreases in Th1-mediated inflammatory cytokines at the site of disease.

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INTERLEUKIN-12 IS A KEY CYTOKINE THAT drives the inflammatory response mediated by type 1 helper T (Th1) cells.^{1,2} As such, it underlies both normal host responses to a variety of intracellular bacterial, fungal, and protozoal pathogens and the abnormal inflammatory responses that accompany many autoimmune diseases, such as Crohn's disease. Crohn's disease is characterized by increased production of interleukin-12 by antigen-presenting cells in intestinal tissue and interferon- γ and tumor necrosis factor α (TNF- α) by intestinal lymphocytes and macrophages.³⁻⁷ These inflammatory cytokines induce and sustain the granulomatous inflammation and bowel-wall thickening that are hallmarks of Crohn's disease.

Targeting interleukin-12 with antibodies is an effective treatment for the intestinal inflammation in animal models of Crohn's disease. Mice with trinitrobenzene sulfonate-induced colitis have a Th1-mediated gut inflammation characterized by greatly increased production of interleukin-12, interferon- γ , and TNF- α . In mice, administration of a monoclonal antibody against interleukin-12 (anti-interleukin-12) can result in the resolution of established colitis and, if given at the time of induction of colitis, can prevent inflammation.⁸ Anti-interleukin-12 can also prevent and treat the spontaneous colitis seen in models of Th1-mediated inflammation such as mice that overexpress the human CD3 ϵ gene and mice deficient in interleukin-10.^{9,10}

We conducted a multisite, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of anti-interleukin-12 for Crohn's disease. We determined the rates of remission, clinical response, and adverse events using two doses and two dosing schedules and measured changes in the secretion of cytokines by mononuclear cells of the colonic lamina propria (LPMCs) after anti-interleukin-12 treatment.

METHODS

PATIENTS

Eligible male or female patients were at least 18 years old, had received a diagnosis of Crohn's disease, and had a score on the Crohn's Disease Activity Index (CDAI) of 220 to 450 within two weeks before beginning treatment (CDAI scores can range from 0 to 600, with higher scores indicating more severe disease).¹¹ Eligible patients could continue to take concomitant medications if such therapy had begun at least 2 weeks before study treatment

in the case of antibiotics; at least 4 weeks before in the case of mesalamine, sulfasalazine, prednisone (20 mg per day or less), or prednisone equivalent; and at least 12 weeks before in the case of azathioprine or mercaptopurine. The doses of these medications had to remain stable throughout the treatment period. Patients who had received antibody against TNF- α , methotrexate, cyclosporine, tacrolimus, thalidomide, or mycophenolate mofetil within four months before randomization were excluded, as were patients who had received any experimental agent or more than 20 mg of prednisone or prednisone equivalent per day within four weeks before randomization and patients who had received corticosteroid or mesalamine enemas within seven days or nonsteroidal antiinflammatory drugs within 24 hours before randomization. Female patients were required to use two forms of contraception throughout the study period. Other exclusion criteria included the presence of an ostomy, intestinal resection resulting in the short-bowel syndrome, a clinically significant abnormality on chest x-ray film or electrocardiogram, bowel obstruction or a known high-grade stricture, probable requirement for intestinal surgery within 12 weeks after randomization, stool examination or culture positive for pathogens or *Clostridium difficile* toxin, Cushing's syndrome, active acute infection requiring antibiotics, clinically significant laboratory abnormalities, active hepatitis B or C virus infection, seropositivity for the human immunodeficiency virus, a history of cancer, and a history of anaphylactic reaction to anti-TNF- α therapy. Women who were pregnant or breast-feeding were excluded. Patients were also excluded if they had a history of tuberculosis, positive purified protein derivative test, receipt of bacille Calmette-Guérin vaccine, or moderate or severe persistent asthma.

Patients were screened for eligibility at participating sites after the protocol had been approved by local institutional review boards or ethics committees. Eligible patients were randomly assigned to receive treatment at 15 centers in the United States, Germany, and the Netherlands from October 2000 to January 2002.

STUDY DESIGN

The study was a multicenter, randomized, placebo-controlled, double-blind, phase 2 clinical trial. After providing written informed consent, patients entered a 14-day screening phase to determine eligibility and pretreatment measurements. Two cohorts

were then enrolled sequentially: Cohort 1 received one injection followed four weeks later by one injection per week for six weeks, and Cohort 2 received one injection per week for seven weeks. On the basis of pharmacokinetic and preclinical data showing that 1 μg of anti-interleukin-12 per milliliter of serum blocked 90 percent of interleukin-12-induced neopterin release in vivo, an equivalent dose of 1 mg per kilogram of body weight was chosen as the lower-limit dose. The four-week interval between the first and second dose in Cohort 1 was chosen to assess the safety of a single dose of anti-interleukin-12 in patients with Crohn's disease. Patients were randomly assigned to receive subcutaneous placebo, anti-interleukin-12 at a dose of 1 mg per kilogram, or anti-interleukin-12 at a dose of 3 mg per kilogram in a 1:2:2 ratio by means of an independent, computer-generated randomization schedule without stratification or block allocation. Patients in Cohort 1 were seen two weeks after the first injection and at weekly intervals coinciding with the next six injections; patients in Cohort 2 were seen weekly during the seven-week treatment phase. All patients were followed for 18 weeks after the final injection of study drug and were seen at 6, 12, and 18 weeks.

The anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) is a recombinant, exclusively human-sequence, full-length IgG₁ λ antibody genetically modified to recognize interleukin-12 p40 protein. The antibody was supplied as a lyophilized powder and reconstituted with water to yield an isotonic solution. The placebo was the same isotonic solution administered in a volume appropriate to the assigned dose.

Patients enrolled at the National Institutes of Health (NIH) study site underwent colonoscopy just before the first injection and 48 hours after the final injection of study drug. At these times, biopsy samples were obtained within the same gut regions only from areas with endoscopically apparent inflammation or ulcer borders; biopsy specimens were used for histologic analysis and for the preparation of LPMCs for cytokine measurements.

All NIH investigators had complete access to the study data for review and analysis. The authors analyzed the data and wrote the article. The study sponsors (Wyeth and Abbott Laboratories) underwrote the costs of the study and measured the serum levels of antidrug antibodies and anti-interleukin-12. The sponsors were not involved in the decision to publish the results.

SAFETY ASSESSMENT

The primary objective of the study was the safety of anti-interleukin-12 treatment in patients with Crohn's disease. Changes in clinical, biochemical, and hematologic variables were assessed on days 15, 29, 43, and 64 in Cohort 1 and on days 8, 22, and 43 in Cohort 2 during the treatment phase and at each visit during the follow-up phase. The severity and cause (study drug or procedure) of adverse events were determined.

EFFICACY ASSESSMENT

Secondary outcomes included measurement of the rates of response and remission at two prespecified points: at the end of treatment (day 64 for Cohort 1 and day 43 for Cohort 2) and the end of follow-up (week 18). Remission was defined by a CDAI score of 150 or less, and a clinical response by a decrease in the CDAI score of at least 100 points. The CDAI was measured before treatment; during the week preceding days 15, 29, 43, and 64 in Cohort 1 and days 8, 22, and 43 in Cohort 2; and 6, 12, and 18 weeks after the final dose of study drug. Changes in the pretreatment levels of cytokine secretion by LPMCs and in the histologic score were also assessed at the end of treatment in the patients enrolled at the NIH.

ANTIDRUG ANTIBODY ASSESSMENT

Antidrug antibodies were measured in patients before treatment; on days 15, 29, 43, and 64 in Cohort 1 and days 22 and 43 in Cohort 2; and at each follow-up visit. Serum samples (diluted 1:25 and 1:75) were incubated in enzyme-linked immunosorbent assay (ELISA) plates coated with anti-interleukin-12; bound antidrug antibodies were detected by means of biotinylated anti-interleukin-12 and horseradish peroxidase-streptavidin. The operational lower limit of detection of antidrug antibodies was a serum dilution of 1:25, and only samples positive for antidrug antibodies at both the 1:25 and 1:75 dilutions underwent further titrating.

PREPARATION OF LPMCs AND MEASUREMENT OF CYTOKINE RELEASE

LPMCs were prepared according to a modification of a previously described procedure.⁴ Cytokines were measured by ELISA from supernatants of cultured cells after stimulation with antibodies against CD2 or CD3 plus CD28 (T-cell stimulus) and *Staphylococcus aureus* Cowan I, interferon- γ , and CD40 ligand trimer (antigen-presenting-cell stimulus).¹²

STATISTICAL ANALYSIS

The determination of the sample size was based primarily on safety outcomes and the ability to observe adverse events. Given a group of 16 patients, all of whom received the same dose according to the same schedule, the power to observe at least one adverse event, assuming a true rate of adverse events of 5 percent, 10 percent, or 20 percent, was 0.56, 0.82, or 0.97, respectively.

The demographic characteristics of the groups and the rates of adverse events were compared by means of descriptive methods, and significant differences were identified by means of the t-test or Fisher's exact test (all tests were two-tailed). Efficacy evaluation was a secondary aim, and data from all patients who underwent randomization were analyzed according to the intention-to-treat principle. Fisher's exact test was used to compare the remission and response rates between the treatment and placebo groups at the prespecified points (the end of treatment and the end of the 18-week follow-up period) without adjustment for multiple comparisons. Changes in mean cytokine secretion before and after treatment were evaluated by the paired t-test.

RESULTS**DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF THE PATIENTS**

Among 123 patients who were screened, 79 eligible patients were enrolled. The CDAI scores indicated that they had moderately active disease despite the concurrent use of medications for Crohn's disease by 75 percent of patients. Eighty-four percent completed all seven study-drug injections, 87 percent completed at least six, and 66 percent completed the entire protocol, including the 18-week follow-up period (Fig. 1). Demographic characteristics were similar within the two cohorts except that the group given 1 mg of anti-interleukin-12 per kilogram in Cohort 1 had had Crohn's disease for a significantly longer time and had a higher CDAI score than the placebo group and was less likely to be taking 5-aminosalicylate drugs or any medication for Crohn's disease than the group given 3 mg of anti-interleukin-12 per kilogram (Table 1). In Cohort 2, patients in the group given 3 mg per kilogram were more likely than those in the placebo group to be taking concomitant immunomodulatory medications (Table 1).

SAFETY AND ADVERSE EVENTS

The most frequently reported adverse event was a local reaction at the injection site (Table 2). This event was significantly more common among patients who received either 1 mg or 3 mg of anti-interleukin-12 per kilogram (range, 77 to 88 percent) than among patients who received placebo (25 percent). The majority of injection reactions to anti-interleukin-12 were mild (88 percent), and all responded to symptomatic therapy. One patient withdrew from the study owing to a local reaction after each of the first two injections. The incidence of other adverse events that occurred in more than 10 percent of patients was not significantly different among the groups in either cohort (Table 2). There were no serious infections.

Four patients discontinued the study because of adverse events: two in the group given 1 mg of anti-interleukin-12 per kilogram (one had injection-site reactions, and one was given a diagnosis of a small-bowel dysplastic adenoma after receiving four injections) and two in the placebo group (one received a diagnosis of a peritoneal abscess, and one had an increase in the symptoms of Crohn's disease). Nine serious adverse events occurred, none attributed to anti-interleukin-12. Two of the nine serious adverse events occurred in patients in the placebo group. The other seven occurred in patients who received anti-interleukin-12: two had adverse events two to three months after the last dose of drug (one had diarrhea and dehydration and one had migraine and bone pain); one was hospitalized for partial obstruction of the small bowel, which did not recur despite continued administration of drug; two had adverse events related to preexisting neoplastic conditions (skin cancer and dysplastic tubular adenoma); one had substance abuse requiring an evaluation in the emergency room; and one patient's wife became pregnant during the study (a concern because of unknown teratogenic effects).

Clinically significant laboratory abnormalities were noted in 17 patients who received anti-interleukin-12 and 5 patients who received placebo. The most frequent abnormalities were hyperuricemia (uric acid levels were 7.5 to 10.0 mg per deciliter in nine patients who received anti-interleukin-12 and two who received placebo and exceeded 10 mg per deciliter in one patient given anti-interleukin-12), hypoglycemia (in two patients given anti-interleukin-12), hyperamylasemia (in two patients given anti-interleukin-12), and hyperphosphatemia (in

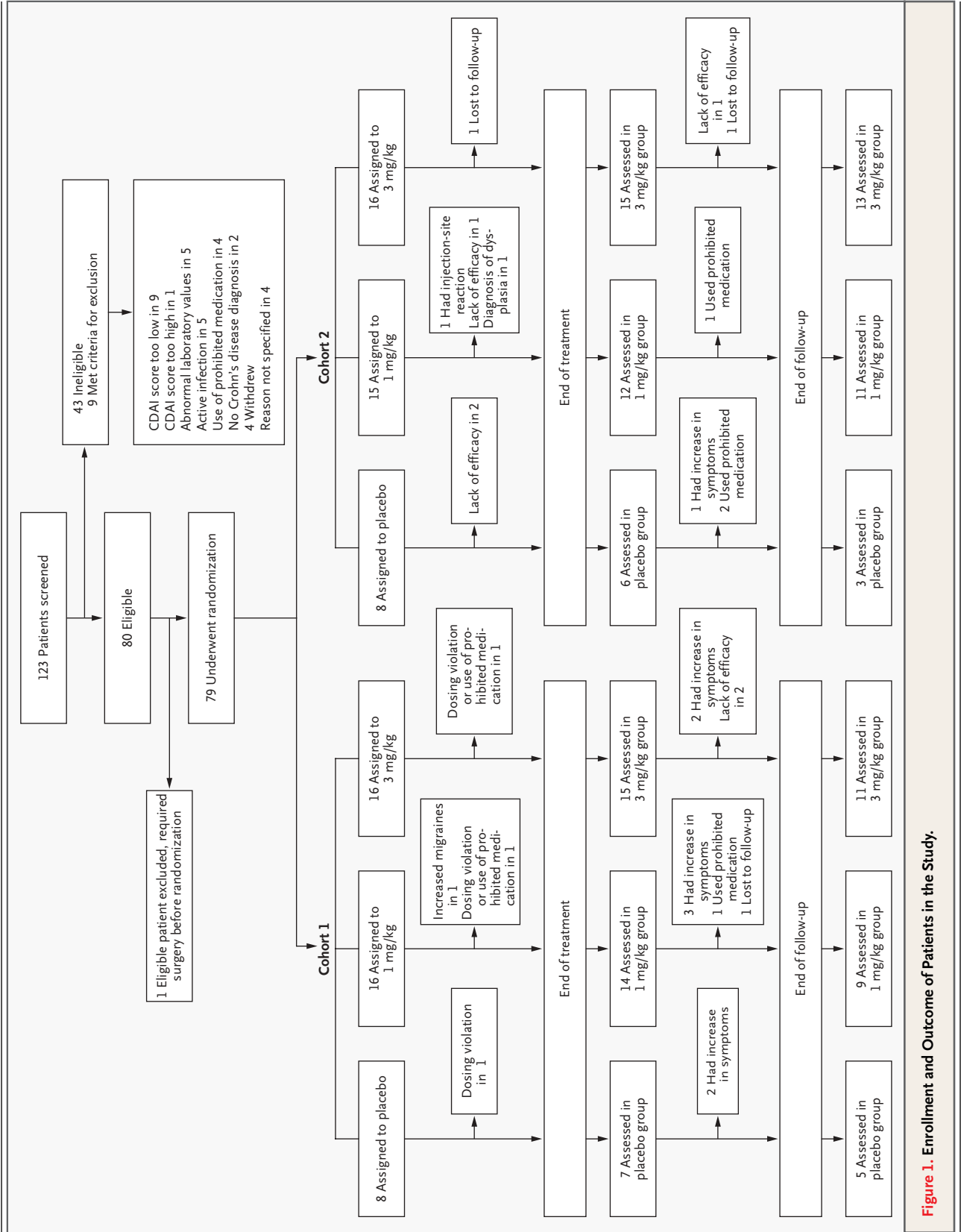


Figure 1. Enrollment and Outcome of Patients in the Study.

Table 1. Baseline Demographic and Clinical Characteristics.*

Characteristic	Cohort 1			Cohort 2		
	Placebo (N=8)	Anti-Interleukin-12 Antibody, 1 mg/kg (N=16)	Anti-Interleukin-12 Antibody, 3 mg/kg (N=16)	Placebo (N=8)	Anti-Interleukin-12 Antibody, 1 mg/kg (N=15)	Anti-Interleukin-12 Antibody, 3 mg/kg (N=16)
Age — yr						
Mean	36.9±13.3	43.1±12.6	42.5±14.2	41±12.6	39.9±13.9	39.8±13.4
Range	20–60	28–80	21–71	23–62	22–72	23–66
Sex — no. (%)						
Male	3 (38)	4 (25)	5 (31)	3 (38)	3 (20)	8 (50)
Female	5 (62)	12 (75)	11 (69)	5 (62)	12 (80)	8 (50)
Weight — kg						
Mean	71.9±11.9	68.8±13.1	68.5±14.5	89.1±25.7	72.5±27.4	67.3±14
Range	54–92	48–91	48–102	55–120	48–131	48–98
Duration of disease — mo						
Mean	76±58	165±145†	139±115	125±117	175±177	154±112
Range	8–181	34–518	15–362	5–305	6–623	12–396
Crohn's Disease Activity Index score‡						
Mean	279±53	360±54†	300±53	335±63	323±54	356±75
Range	231–399	282–469§	204–380¶	235–427	250–440	239–529§
Disease location — no. (%)						
Ileum	4 (50)	3 (19)	4 (25)	4 (50)	4 (27)	3 (19)
Colon	3 (38)	5 (31)	6 (38)	1 (12)	2 (13)	7 (44)
Ileum and colon	1 (12)	8 (50)	6 (38)	3 (38)	9 (60)	6 (38)
Fistulizing disease — no. (%)	3 (38)	10 (62)	7 (44)	3 (38)	6 (40)	10 (62)
Prior bowel resection — no. (%)	4 (50)	4 (25)	4 (25)	3 (38)	5 (33)	7 (44)
Concomitant medication — no. (%)						
None for Crohn's disease	2 (25)	7 (44)	1 (6)	4 (50)	4 (27)	2 (12)
5-Aminosalicylate drugs	6 (75)	7 (44)	14 (88)	3 (38)	7 (47)	10 (62)
Corticosteroids	4 (50)	3 (19)	4 (25)	2 (25)	6 (40)	3 (19)
Immunomodulators	2 (25)	5 (31)	7 (44)	0	4 (27)	8 (50)†

* Plus-minus values are means ±SD.

† P<0.05 for the comparison with the placebo group in the cohort.

‡ Scores for the Crohn's Disease Activity Index can range from 0 to 600, with higher scores indicating more severe disease.

§ Two patients had a baseline score of more than 450, and both were included in the analysis.

¶ One patient had a baseline score of less than 220 and was included in the analysis.

|| P<0.05 for the comparison with the group given 1 mg per kilogram in the cohort.

two patients given placebo). None of these abnormalities required withdrawal from the study.

DEVELOPMENT OF ANTIDRUG ANTIBODIES

Antidrug antibodies were detected in only three patients, all of whom received 1 mg of anti-interleu-

kin-12 per kilogram. Antidrug antibodies developed three to four months after the final injection in two patients in Cohort 1 who had a response. These two patients also had unexpectedly low serum levels of anti-interleukin-12 and early clearance of anti-interleukin-12 from the serum; the

Table 2. Adverse Events Occurring in More Than 10 Percent of Patients and Incidence of Antidrug Antibodies against Anti-Interleukin-12.

Adverse Event	Cohort 1			Cohort 2		
	Placebo (N=8)	Anti-Interleukin-12 Antibody, 1 mg/kg (N=16)	Anti-Interleukin-12 Antibody, 3 mg/kg (N=16)	Placebo (N=8)	Anti-Interleukin-12 Antibody, 1 mg/kg (N=15)	Anti-Interleukin-12 Antibody, 3 mg/kg (N=16)
	<i>number of patients (percent)</i>					
Nausea	2 (25)	3 (19)	2 (12)	1 (12)	0	3 (19)
Vomiting	0	3 (19)	2 (12)	1 (12)	0	3 (19)
Abdominal pain	0	1 (6)	1 (6)	0	1 (7)	2 (12)
Arthralgia	0	0	2 (12)	1 (12)	3 (20)	1 (6)
Urinary tract infection	0	3 (19)	1 (6)	1 (12)	1 (7)	0
Bronchitis	0	0	2 (12)	0	0	2 (12)
Cough	2 (25)	2 (12)	1 (6)	1 (12)	0	0
Headache	1 (12)	6 (38)	1 (6)	1 (12)	1 (7)	5 (31)
Fever	1 (12)	4 (25)	4 (25)	2 (25)	1 (7)	3 (19)
Fatigue	0	2 (12)	1 (6)	2 (25)	1 (7)	2 (12)
Local injection-site reaction	3 (38)	12 (75)	13 (81)	1 (12)	12 (80)*	15 (94)*
Antidrug antibodies against anti-interleukin-12†	0	2 (12)	0	0	1 (7)	0

* P<0.005 for the comparison with the placebo group in the cohort.

† Values reflect the overall incidence of antidrug antibodies.

loss of response coincided with the detection of antidrug antibodies in one patient and preceded the detection of antidrug antibodies by two months in the other patient. Antidrug antibodies were detected before treatment in one patient in Cohort 2 and persisted to the end of follow-up. This finding was not considered to be drug related, because it was present before treatment, there was no change in titer with treatment, and there was no association with low serum levels of anti-interleukin-12. Since the presence of measurable levels of anti-interleukin-12 in serum can interfere with the detection of antidrug antibodies, this assay may have underestimated the incidence of antidrug antibodies. However, only three additional patients who received anti-interleukin-12 had unexpectedly low serum antibody levels, suggesting the presence of antidrug antibodies, but no antidrug antibodies were detected, even when anti-interleukin-12 levels fell below quantifiable levels.

CLINICAL RESPONSE AND REMISSION

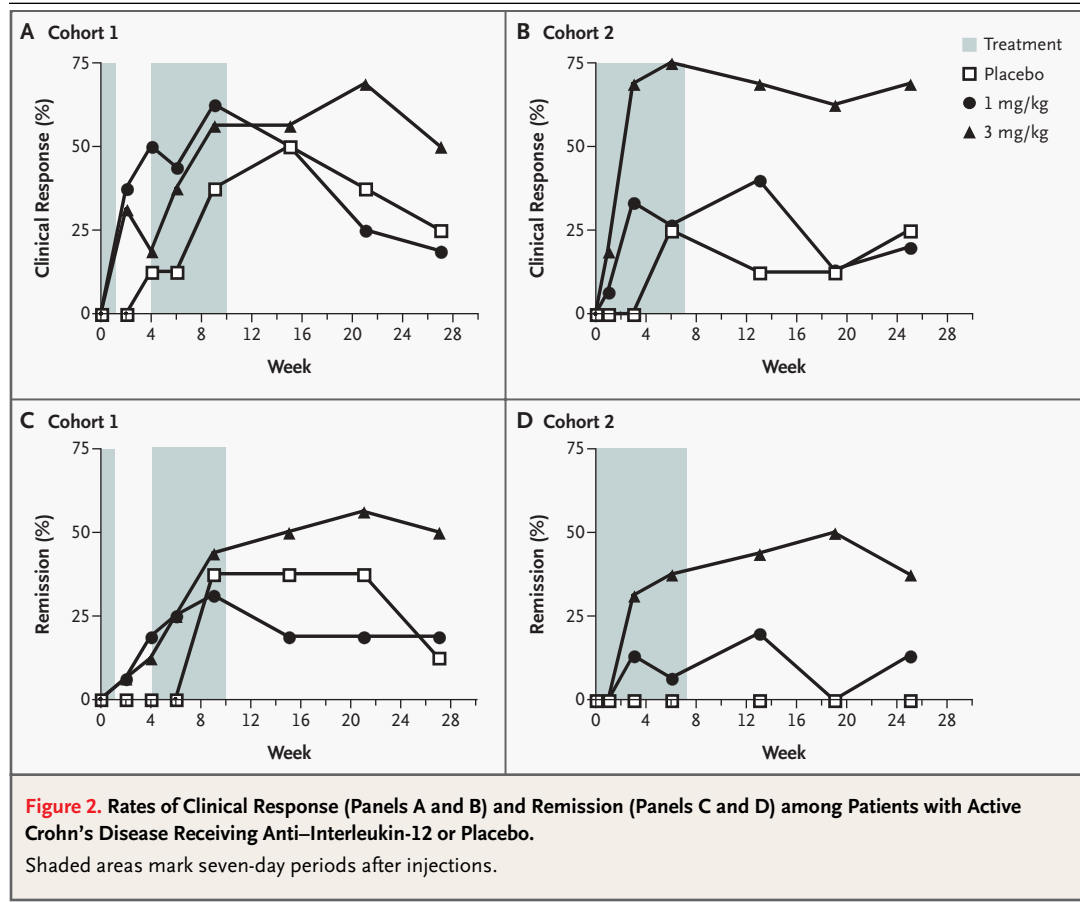
Cohort 1

The Cohort 1 dosing schedule gave us an opportunity to assess the effect of a single dose of anti-interleukin-12 as compared with that of placebo. Four weeks after one injection of study drug, 50 per-

cent of the patients in the group given 1 mg per kilogram had a clinical response, as compared with 19 percent in the group given 3 mg per kilogram and 13 percent in the placebo group (Fig. 2A). The rate of remission at this time was 19 percent in the group given 1 mg per kilogram, 12 percent in the group given 3 mg per kilogram, and 0 percent in the placebo group (Fig. 2C). However, at the time of the final injection (nine weeks), the response rate was 63 percent in the group given 1 mg per kilogram, 56 percent in the group given 3 mg per kilogram, and 38 percent in the placebo group, and the remission rates were 31 percent, 44 percent, and 38 percent, respectively. At the end of the 18-week follow-up phase, the group given 3 mg per kilogram maintained remission and response rates of 50 percent, as compared with 19 percent each in the group given 1 mg per kilogram and 13 percent and 25 percent, respectively, in the placebo group. At no time were the rates in the anti-interleukin-12 groups significantly different from those in the placebo group.

Cohort 2

The Cohort 2 dosing schedule was a continuous induction therapy of seven weekly injections of study drug. At the time of the final injection (seven



weeks), the respective response and remission rates were 27 percent and 8 percent in the group given 1 mg per kilogram, 75 percent and 38 percent in the group given 3 mg per kilogram, and 25 percent and 0 percent in the placebo group (Fig. 2B and 2D). At the end of the 18-week follow-up phase, the respective response and remission rates were 69 percent and 38 percent in the group given 3 mg per kilogram, 20 percent and 13 percent in the group given 1 mg per kilogram, and 25 percent and 0 percent in the placebo group. The response rate was significantly higher in the group given 3 mg per kilogram than in the placebo group at the end of treatment (75 percent vs. 25 percent, $P=0.03$) but not at the end of follow-up (69 percent vs. 25 percent, $P=0.08$). Remission rates in the group given 3 mg per kilogram did not differ significantly from those in the placebo group at either the end of treatment or the end of follow-up (38 percent at both times vs. 0 percent at both times, $P=0.07$).

EFFECTS OF ANTI-INTERLEUKIN-12 ON CYTOKINE SECRETION BY LPMCs AND HISTOLOGIC FINDINGS

Treatment with anti-interleukin-12 was associated with decreases in the secretion of interleukin-12, interferon- γ , and TNF- α by LPMCs after in vitro T-cell stimulation (used for interferon- γ and TNF- α) and antigen-presenting-cell stimulation (used for interleukin-12) (Fig. 3). Although the secretion of interleukin-6 also decreased after antibody treatment ($P<0.06$), the changes in the secretion of interleukin-10 and interleukin-18 were not significant. The magnitude of change in cytokine secretion did not correlate with the dose or regimen of anti-interleukin-12, but the only antibody-treated patient in this subgroup who did not have a clinical response (Patient 8 in Fig. 3) had an increase in interferon- γ secretion and no change in TNF- α secretion. One patient in the subgroup (Patient 2) (data not shown) was randomly assigned to receive placebo but in-

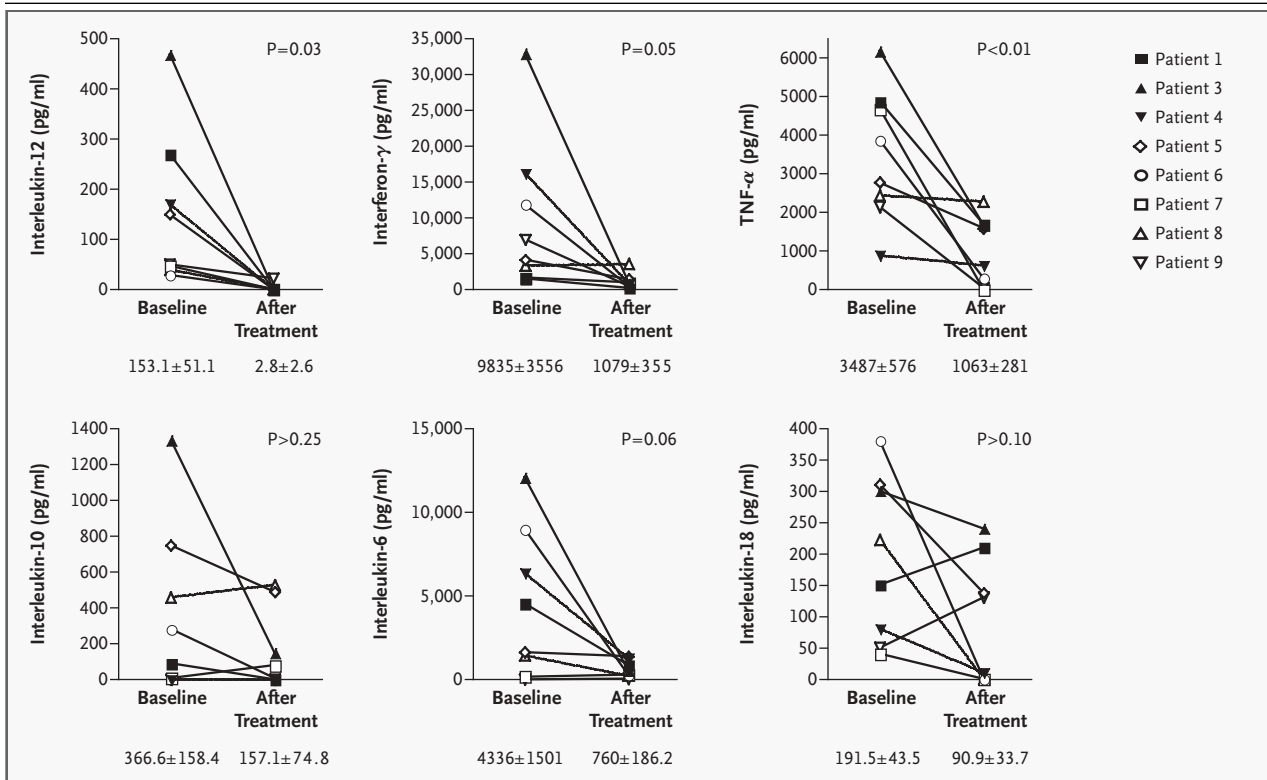


Figure 3. Cytokine Secretion by Mononuclear Cells of the Colonic Lamina Propria from Patients with Crohn's Disease before and after Treatment with Anti-Interleukin-12.

The mean (±SD) levels of each cytokine before and after treatment are shown under the x-axis. Solid symbols indicate patients in Cohort 1, and open symbols patients in Cohort 2.

advertently received a single dose of antibody as the fifth scheduled dose. This patient's baseline secretion of interleukin-12 (310 pg per milliliter), interferon-γ (18,515 pg per milliliter), and TNF-α (6468 pg per milliliter) fell to 0, 1471, and 4342 pg per milliliter, respectively, two weeks later, coinciding with the induction of remission (reflected by a decrease in the CDAI score from 279 to 143).

Treatment with anti-interleukin-12 in this subgroup of patients was also associated with a decrease in mucosal histologic abnormalities. Of the eight patients in the subgroup who received anti-interleukin-12, seven had a response, and six of these seven had improved mucosal histologic scores. Using a modified d'Haens scoring system (no points were assigned for number of affected biopsy samples, so the maximal possible score was 13 instead of 16),¹³ we found that the mean (±SD) score in these seven patients was 6.9±3.8 before treatment and 3.6±2.1 at the end of treatment

(P=0.06). The one patient who did not have a response to antibody treatment and who had no decrease in cytokine secretion had an increase in the histologic score. The patient in the placebo group who received a single dose of anti-interleukin-12 two weeks before the end-of-treatment biopsies also had an increase in the histologic score; this change is consistent with the patient's worsening symptoms just before the single dose of antibody and notable in the light of the decrease in cytokine secretion by LPMCs two weeks later. In general, histologic improvement was characterized by decreased numbers of neutrophils, lymphocytes, and plasma cells and reduced epithelial damage, with the reappearance of goblet cells.

DISCUSSION

Our data demonstrate that targeting interleukin-12 p40 with a specific antibody may induce a clini-

cal response or remission in patients with active Crohn's disease. Furthermore, the resulting clinical responses and remissions could be rapid in onset and durable. Patients treated with 3 mg of anti-interleukin-12 per kilogram had clinical responses after three weekly injections, and the response was sustained for at least 18 weeks after treatment.

The uninterrupted series of weekly injections of 3 mg per kilogram (in Cohort 2) resulted in higher response rates than the interrupted series (in Cohort 1). The difference in treatment effects between the regimens may be related to the speed with which maximal serum levels of anti-interleukin-12 are attained or sustained. In Cohort 1, the mean anti-interleukin-12 serum level was 1264 ± 1675 ng per milliliter before the injection at week 4, as compared with 8271 ± 2523 ng per milliliter before the injection at week 3 in Cohort 2, whereas the respective end-of-treatment levels of anti-interleukin-12 were similar ($10,022 \pm 5807$ ng per milliliter in Cohort 1 and 9374 ± 2898 ng per milliliter in Cohort 2). In addition, the response and remission rates in the placebo group in Cohort 2 were lower than those in the placebo group in Cohort 1. Because of the intention-to-treat analysis used, the curves for the placebo group in Cohort 1 included the remission in the patient who mistakenly received a single dose of 1 mg of anti-interleukin-12 per kilogram. On average, the placebo group in Cohort 2 had had Crohn's disease longer and had higher baseline CDAI scores than the placebo group in Cohort 1, differences that may have contributed to the lower remission rates (but possibly higher response rates¹⁴) in this group. The placebo group in Cohort 2 also had a lower rate of use of medications for Crohn's disease.

With the exception of local reactions at injection sites, there were no significant differences in the rate of adverse effects between placebo and anti-interleukin-12. For the most part these skin reactions were transient and mild and did not require treatment, and subcutaneous injection is a less costly and less time-consuming approach than intravenous infusion. Injection-site reactions occurred more frequently among patients who had a response to anti-interleukin-12 than among patients who did not have a response (44 of 52 [85 percent] vs. 7 of 11 [64 percent]), but the difference was not significant ($P=0.20$). Although the incidence of infections was not significantly increased in the anti-interleukin-12 group as a whole, the risk of infection with longer-term use remains to be established.

Evaluation of the cytokine responses before and soon after treatment showed that the secretion of interleukin-12, interferon- γ , and TNF- α by colonic LPMCs was notably decreased. Thus, targeting interleukin-12 can also reduce the levels of downstream effectors such as interferon- γ and TNF- α . However, it is important to note that since the anti-interleukin-12 that we used recognizes the interleukin-12 p40 chain, interleukin-23 — another Th1-inducing cytokine that shares the p40 chain — may also be involved in the observed responses.^{15,16} The reduced secretion of interleukin-12 after treatment with anti-interleukin-12 suggests that treatment decreased the number of interleukin-12-producing macrophages¹⁷ or that reduced secretion of interferon- γ eliminates the enhancing effect interferon- γ exerts on the secretion of interleukin-12 by macrophages.^{18,19} In addition, response and remission rates did not appear to depend on increasing the secretion of interleukin-10, an antiinflammatory cytokine that is thought to play a role in regulating gut inflammation.²⁰

The data from this early phase 2 study provide some evidence that an antibody targeted to interleukin-12 p40 is active against the inflammation of Crohn's disease as well as show that treatment with this agent may induce clinical response and remission. The clinical effects of anti-interleukin-12 in patients with Crohn's disease suggest that interleukin-12 has an important role in the ongoing inflammatory reaction of Crohn's disease, even in long-standing disease. Furthermore, these preliminary data will help guide the design of future studies to assess the efficacy of anti-interleukin-12 in Crohn's disease and, by extension, other Th1-mediated inflammatory disorders.

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Drs. Fuss, Neurath, and Strober report being coholders of a patent for the use of anti-interleukin-12 in Crohn's disease. Dr. Neurath reports having served as a speaker for Novartis. Dr. Mayer reports having served as a speaker for Centocor and a consultant for Therakos. Dr. Elson reports having served as a consultant for Corixa, Abbott, Solvay, and AstraZeneca and having received grant support from Sankyo. Dr. Sandborn reports having served as a consultant for Abbott, Ajinomoto Pharmaceuticals, Amgen, AstraZeneca, Atrix Laboratories, Berlex, Boehringer Ingelheim, Celltech, Centocor, Elan, Chemocentryx, Chugai, Combinatorx, Genentech, GlaxoSmithKline, H3 Pharma, Hoffmann-La Roche, Isis Pharmaceuticals, McNeil Consumer and Specialty, Merck, Millennium, Novartis, Ono Pharmaceuticals, Otsuka, Pharmadigm, Procter & Gamble, Prometheus, Protein Design Labs, Salix, Sangstat, Schering Canada, Serono, Shire, Targacept, Teva, Therakos, Tillott's Pharma, and Vela Pharmaceuticals; having served as a paid speaker for Abbott, AstraZene-

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APPENDIX

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CORRECTION

Anti-Interleukin-12 Antibody for Active Crohn's Disease

Anti-Interleukin-12 Antibody for Active Crohn's Disease . On page 2069, the list of authors omitted the name of Zhiqiong Yang, B.S., of the Mucosal Immunity Section, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Md.